Year: 2018

Facial soft tissue growth in identical twins

Hersberger-Zurfluh, Monika A ; Papageorgiou, Spyridon N ; Motro, Melih ; Kantarci, Alpdogan ; Will, Leslie A ; Eliades, Theodore

Abstract: INTRODUCTION: The aims of this longitudinal analysis of untreated monozygotic twins were to investigate the change of the facial soft tissues during growth, to determine the concordance of soft tissue growth patterns between genetically identical twins, and to assess the genetic component of soft tissue development. METHODS: The sample consisted of 33 pairs of untreated monozygotic twins (23 male, 10 female) from the Forsyth Moorrees Twin Study (1959-1975); lateral cephalograms taken from 6 to 18 years of age were analyzed at 3-year intervals. Cephalograms were traced, and longitudinal changes in the soft tissue profile between twins were analyzed with intraclass correlation coefficients and linear regression modelling. RESULTS: The concordance between monozygotic twins at 18 years of age was moderate to high with intraclass correlation coefficients values between 0.37 and 0.87. Additionally, female twins showed higher concordance at 18 years of age than did male twins for all included variables. However, about 10% to 46% of the twin pairs had large differences in their soft tissue parameters, even after the growth period. CONCLUSIONS: Although monozygotic twins possess the same genetic material, differences in the soft tissues were found. This supports the complex developmental mechanism of the human face and the varying influence of genetic and environmental factors.

DOI: https://doi.org/10.1016/j.ajodo.2018.01.020

Originally published at:
Hersberger-Zurfluh, Monika A; Papageorgiou, Spyridon N; Motro, Melih; Kantarci, Alpdogan; Will, Leslie A; Eliades, Theodore (2018). Facial soft tissue growth in identical twins. American Journal of Orthodontics and Dentofacial Orthopedics, 154(5):683-692.
DOI: https://doi.org/10.1016/j.ajodo.2018.01.020

Facial Soft Tissue Growth in Identical Twins

Monika A. Hersberger-Zurfluh, DMD, Dr med dent,[a,b] Spyridon N. Papageorgiou, DDS, Dr med dent,[a] Melih Motro, DDS, PhD,[c] Leslie A. Will, DMD, MSD,[d] and Theodore Eliades, DDS, MS, Dr Med Sci, PhD, FIMMM, FRSC, FInstP[e]

Zurich, Switzerland, and Boston, Mass

[a]Senior Teaching and Research Assistant, Clinic of Orthodontics and Pediatric Dentistry, Center of Dental Medicine, University of Zurich, Zurich, Switzerland.

[b]Clinical Associate, Department of Orthodontics & Dentofacial Orthopedics, Boston University Goldman School of Dental Medicine, Boston, Mass.

[c]Clinical Assistant Professor, Department of Orthodontics & Dentofacial Orthopedics, Boston University Goldman School of Dental Medicine, Boston, Mass.

[d]Anthony A. Gianelly Professor and Chair, Department of Orthodontics & Dentofacial Orthopedics, Boston University Goldman School of Dental Medicine, Boston, Mass.

[e]Professor and Director, Clinic of Orthodontics and Pediatric Dentistry, Center of Dental Medicine, University of Zurich, Zurich, Switzerland.

All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and none were reported.

Address correspondence to: Leslie A. Will, Department of Orthodontics, Henry M. Goldman School of Dental Medicine, Boston University, 100 E Newton St, Boston, MA 02118; e-mail, willla@bu.edu.

Financial Disclosure: All authors have no financial relationships relevant to this article to disclose.

Abstract

Introduction: The aim of this longitudinal analysis of untreated monozygotic twins was to investigate the change of the facial soft tissues during growth, to identify distinct facial patterns, and to assess the genetic component of soft tissue development. **Methods:** The sample consisted of 33 pairs of untreated monozygotic twins (23 male, 10 female) from the Forsyth Moorrees Twin Study (1959-1975), where lateral cephalograms taken from 6 to 18 years of age were analyzed in three-year intervals. Cephalograms were traced and longitudinal changes in the soft tissue profile between twins were analyzed with Intraclass Correlation Coefficients (ICC) and linear regression modelling. **Results:** The concordance between monozygotic twins at 18 years of age was moderate to high with ICCs ranging between 0.37 and 0.87. Additionally, female twins showed higher concordance at 18 years of age than male twins for all included variables. However, about 10%-46% of the twin pairs had large differences in their soft tissue parameters, even after the growth period. **Conclusions:** Although monozygotic twins possess the same genetic material, differences in the soft tissues were found. This supports the complex developmental mechanism of the human face and the varying influence of genetic and environmental factors.

INTRODUCTION

Facial morphology and attractiveness have a direct impact on social interactions, psychological development, and other aspects of personal or professional life.[1] Therefore, the development of the human face during the growth period and the attempt to influence it[2] have attracted considerable interest in orthodontics and dentofacial orthopedics.[3]

Research on the dynamic and complex phenomena of growth and development of the facial soft tissues has traditionally been conducted on photographs, anteroposterior or lateral cephalograms or three-dimensional facial scans.[4-6] Growth of the human face, although initially considered to be mainly driven by the growth of the craniofacial bones,[7-9] is complex, with various facial components developing, proportionally, disproportionally, or independent to change of the underlying bones.[10]

The craniofacial complex is derived from a complex developmental process, where gene expression and molecular interactions play an early embryonal role, whilst hormonal and biomechanical environmental factors act mainly during the later childhood and pubertal growth.[11-14] Important insights in the relative contribution of genetic and environmental factors might be acquired from studying human twins, since they share all or part of their genome,[15] thus enabling partitioning of genetic and environmental components.

An early study with twins and same-sex singletons indicated that many cephalometric variables are under strong genetic control, especially those pertaining to the vertical dimension, and that heritability is stronger in the anterior than in the posterior craniofacial region.[16] This strong genetic influence on the vertical dimension of the face was confirmed in a subsequent study of Chinese female twins, which indicated that early orthodontic intervention would be better aimed towards the anteroposterior than the vertical dimension.[17] This seems, however, to be refuted by a later study on twins that indicated that although the facial profile resemblance among twins was high, the shape and sagittal position of the mandible is under stronger genetic control than is its size and vertical relationship to the cranial base.[18] A recent cross-sectional study on female twin patients found that genetic factors account for more than 70% of the phenotypic variation of the size of the face, nose, lip prominence, and the interocular distance.[19] Finally, it was reported that although size of the face showed signs of potential dominant genetic influence, facial proportions were influenced more by environmental factors.[20]

Variations in the facial proportions through time using longitudinal growth data have been previously reported, [21, 22] but these can be attributed to a combination of both genetic and environmental factors, since unrelated patients were studied. However, to our knowledge the soft tissue profile has not yet been assessed using monozygotic twins, which per definition share their genetic material. Therefore, the aim of this retrospective longitudinal cephalometric cohort study was to assess the changes in the facial soft tissues during childhood, adolescent, and early adult growth of untreated monozygotic twins in order to (i) determine the concordance of soft tissue growth patterns between genetically identical twins and to (ii) to assess possible differences between them as a proxy for the genetic / environmental contribution to facial soft tissue development.

MATERIAL AND METHODS

Patient sample

Patients for this retrospective cohort study were recruited from the Forsyth Moorrees Twin Study that was performed during 1959-1975 at the Forsyth Infirmary for Children in Boston after appropriate Institutional Review Board approval (Boston University #H-31945). The original sample contains records from over 500 families of twins with no previous history of orthodontic treatment. Eligible patients for this study were those with (i) Caucasian origin, (ii) no history of orthodontic treatment, craniofacial anomalies, or chronic systemic disease and (iii) available lateral cephalograms in good quality with the soft tissue profile clearly discernable. All patients were measured at approximately the same time points every 3 years from middle childhood to early adulthood: T1 at 6 years, T2 at 9 years, T3 at 12 years, T4 at 15 years, and T5 at 18 years of age.

Cephalometric analysis of facial soft tissue parameters

Six widely used soft tissue measurements were made on the films of 33 pairs of twins at five time points (T1-T5), including facial convexity angle, nasal prominence, nasolabial angle, upper lip length, upper lip thickness, and soft tissue chin thickness (Fig 1). As the aim of this study was to assess the concordance between twins, the analysis was based on the difference between the two twins within a pair instead of overall facial convexity and its change or malocclusion type.

All lateral cephalograms were taken with the same device (copy of the Broadbent cephalometer) in a standardized position in centric occlusion and the head aligned in natural head position. This position was stabilized with ear rods and a nasal support to prevent variations in the head position. The focus-coronal plane distance was 9 cm,[23] and the film-coronal plane distance was 150 cm, which resulted to a constant magnification factor of 6%. The subjects were asked to refrain from swallowing during the radiological examination, with tongue posture subsequently assessed on the cephalograms to ascertain that no children swallowed during the radiographic examination.

After anonymization of all documents with a unique code, radiographs were traced by one person (MHZ) using the Dolphin software (Dolphin Imaging and Management Solutions, Chatsworth, CA).

Sample size calculation

Sample size calculation was performed *a priori* and aimed to find a clinically significant concordance in the primary outcome (facial convexity) between monozygotic twins with the Intraclass Correlation Coefficient (ICC). Based on previous data,[24] we assumed an ICC=0.50 between twins of each pair at T5 and aimed to find a minimal statistically significant difference of half a Standard Deviation (SD) with a paired t-test. Assuming a change of 2° in facial convexity, with a SD of 4° for T5 from a similar study,[25] α=5%, and power=80% it was calculated that a sample of 28 twin pairs was needed, to which 5 more twin pairs were added to account for any missing patient files.

Statistical analysis

Descriptive statistics (means and SD) were calculated for all variables. For each cephalometric variable the concordance of monozygotic twins after growth cessation (T5 – 18 years) was assessed by calculating ICCs and their 95% CIs.

Additionally, the absolute difference of each cephalometric variable within each twin pair was calculated. We also conducted mixed-effects linear regressions to calculate if sex and sagittal jaw relationship (ANB angle) were associated with considerable soft tissue differences between twins, while accounting for repeated measurements (time-points T1 through T5) per patient. The regression results were expressed as unstandardized regression coefficients (β) and their 95% CIs.

Finally, in order to identify twins with considerable differences in their soft tissue parameters, the percentage of twin pairs that had absolute difference between twins greater than one SD for each variable was calculated. Sex, sagittal jaw relationship (ANB angle), and growth through T1-T5 were again tested for association with considerable soft tissue differences through mixed-effects binomial regressions. The regression results were expressed as Relative Risks (RR) and their 95% CIs.

All analyses were run in Stata SE 14.0 (StataCorp LP, College Station, TX) with an unadjusted α of 5%, since the study's scope is based on descriptive analysis of concordance and associated factors.

Repeatability and sensitivity analysis

A random sample of 180 cephalograms was chosen and re-measured from the same assessor (MHZ) after one month for repeatability. The repeatability/agreement of the repeated measurements were assessed with the Concordance Correlation Coefficient[26] and the Bland- Altman method, [27] while the error of the method was calculated with Dahlberg's formula.[28]

RESULTS

A total of 33 eligible monozygotic twin pairs (66 twins) were included in the present study. From those 33 twin pairs, 23 (70%) were male and 10 (30%) were female. At T1, while 2 pairs of twins were skeletal Class I (defined as having an ANB > 0 but ≤ 4°), 24 pairs were skeletal Class II and 7 pairs were discordant, with 6 pairs having 1 twin Class I and 1 twin Class II, and 1 pair having a mixture of Class I and III. However, by T5, the distribution had changed, with 2 pairs having skeletal Class III relationships, 3 mixing Class I and III in the pair, 5 having Class I, 12 mixing Class I and II, and 11 pairs being both skeletal Class II. Some radiographs were missing at some time points or were of poor quality, which resulted to a total of 287 lateral cephalograms, with varying sample size throughout the study period.

Concordance between twins

A concordance between monozygotic twins at 18 years (T5) could be seen, but varied considerably among the included variables, with facial convexity being the most concordant (ICC=0.87), followed by nasal prominence (ICC=0.68), nasolabial angle (ICC=0.70), upper lip length (ICC=0.69), upper lip thickness (ICC=0.54), and soft tissue chin thickness (ICC=0.37) (Table I). Additionally, a consistent difference between female and male twins was seen, where female twins showed higher concordance at T5 than male twins for all included variables. Overall, the results indicate that the similarity of monozygotic twins in the soft tissue profile in early adulthood varies among the several variables that were studied.

Differences between twins

The descriptive statistics about the average values and the mean difference between twins for each variable at each time point can be seen in Table II. Overall, no clear pattern could be seen for differences between twins of each pair from 6 to 18 years (T1-T5). The regression modeling of the differences between twins and their variation during growth showed that no clear pattern could be found for most of the outcomes (Table III) (Figs. 2-4). The only exception was the nasolabial angle where difference between twins decreased at 15 years compared to 6 years (b=-2.85°; 95% CI=-5.69 to 0; P=0.05). Additionally, differences in the nasolabial angle between twins were associated with sagittal jaw relationship, with larger ANB values leading to larger differences in nasolabial angle between twins (b=0.54; 95% CI=0.14 to 0.93; P<0.05). Finally, sex was significantly associated with difference in upper lip length between twins, where male twins had larger differences in upper lip length than female twins (b=0.92 mm; 95% CI=0.11 to 1.74; P<0.05).

The number of twin pairs with considerable differences (greater than 1 SD) in soft tissue variables can be seen in Table IV. The percentage of twin pairs ranged from 10% to 46% for the various outcomes with no differences across time points. The only exception was the nasolabial angle, where the differences between twins tended to decrease with time (P= 0.022). This was also confirmed by the results of the binominal regression (Table V), where twins at T4 were 77% less likely to have considerable different nasolabial angle (RR=0.23; 95% CI=0.07,0.72; P=0.012) compared to T1 (Table V). Additionally, male twin pairs were considerably more likely to have considerably different upper lip length between the two twins than female twin pairs (RR=3.36; 95% CI=1.21, 9.34; P=0.020).

Repeatability and sensitivity analysis

The analysis of the repeated measures indicated excellent reliability both with high overall CCC of 0.998 (95% CI=0.997, 0.998; P<0.001), low overall Dahlberg's errors (2.41° and 1.10 mm for angular and linear measurements, respectively), and consistent results from the Bland-Altman method for each outcome.

DISCUSSION

This study assessed longitudinally the facial profile of 33 pairs of untreated monozygotic twins from 6 to 18 years of age as seen on lateral cephalograms. A complex facial variation pattern through the growth period can be seen, which seems to be influenced by the patient's sex and the sagittal jaw discrepancy, although not uniformly for all facial components.[29] The overall craniofacial development pattern (for example short- or long-face) seems to be established early and remain relative stable during adolescent and subsequent growth.[30] In contrast, nasal prominence, as well as thickness and length of the upper lip, seem to show an increase through adolescence according to longitudinal growth studies[8,25,31,32,33] and previous cross-sectional studies from adolescence to adulthood on non-related patients[34,35], although issues existed in the statistical analyses of some of these studies, which did not properly account for the longitudinal nature of the data.[36]

Interestingly, the concordance between twins in this study was variable and even though the facial variables of the twins were more consistent after growth cessation at T5 with concordance ranging from 37% to 87% (Table I), a relatively large proportion of the twin pairs had considerable differences in their soft tissue measurements (Table IV). This might indicate that room for individual variation exists even between genetic identical twins and environmental factors might influence the development of the soft tissues. Additionally, the variation in some soft tissue components, such as the upper lip length, showed a statistically significant difference between sexes, with female twins being more consistent than male twins. However, the monozygotic twins that were included in the present study lived in the same families and conditions and therefore the differences in environmental influence can be expected to be small.[37] Therefore, the explanation for this could be found in a stronger genetic component for this trait in female patients.

It should also be noted that craniofacial growth cessation takes place earlier for female than for male subjects[38] with male subjects developing both 2 years longer and therefore relatively more than female subjects.[39] The same is true for the nasal profile, where, increments in nasal height, depth, and inclination are mostly stable in female subjects by 16 years of age, while they continue to increase for male subject up to and after 18 years of age.[40] Therefore, it comes to no surprise that upper lip length was greater in males than females (Table III), as the former continue to grow beyond T5. Likewise, this difference in growth cessation might explain why female twins were more generally more concordant than male twins at T5 (Table I), since a greater proportion of the latter is still in active growth phase.

In any case, the concordance between the soft tissue profiles of twins at the end of the study period (T5) was moderate to high for most of the studied variables (Table I). This is on par with previous cross-sectional non-twin studies on siblings that indicate that the heritability of skeletal craniofacial variables increases with age.[41] The twin concordance in the present study was highest with 87% for facial convexity, followed by 70% and 68% for the nasolabial angle and nasal prominence, respectively. This might be related to the strong genetic component of the nose, something that seems to be confirmed by genome-wide association analyses.[42] As monozygotic twins are considered to possess 100% identical genetic material, it might be assumed that high concordance rates for a specific soft tissue measurement indicate that this trait is determined to a higher degree genetically and less by environmental factors. A previous twin study on several skeletal cephalometric variables indicated high heritability, with vertical skeletal variables showing more heritability than horizontal ones[20,42] and that the lower third of the face seems to be under strong genetic control.[43-45] Additionally, a cross- sectional study on non-twin siblings indicated that the vertical face proportions showed moderate heritability and soft tissues like the soft tissue facial angle or the soft tissue chin thickness had rather strong genetic influences.[46] By contrast, the lips – and especially the upper lip - were heavily influenced by environmental factors.

The cursory evaluation of changes in sagittal jaw relations gave results that were often misleading. With the boundary between Class I and Class II being 4° and the border between Class I and Class III being 0°, changes of a few tenths of a degree sometimes changed the classification of the patient from one class to another and changed the perception of concordance. In general, however, there was a trend towards a decrease in ANB across all the subjects, with fewer Class II subjects and more Class III subjects at the end of growth.

It should be recognized that although the children included in this sample were said to be free from craniofacial anomalies and systemic disease, the recognition of many disorders, including habits, allergies, and airway disturbances, may not have occurred, which could conceivably have influenced their facial growth. There was no access to medical records 50-60 years after the records were taken, so the presence or absence of multiple conditions that could influence growth cannot be verified.

The minimization of genetic variation by including monozygotic twins and the longitudinal assessment of facial changes during a large portion of the growth period can be counted among the strengths of this study. However, some limitations exist. Any variation measured during the study period is a sum of growth, environmental influences, and random error. Additionally, missing or low quality radiographs at certain time-points led to sample reduction and a subsequent loss of power. Finally, all patients were Caucasian, which might preclude generalization to other patient populations.

CONCLUSIONS

The present longitudinal study on monozygotic twins through growth indicates that:

1. Facial profile develops in a complex way with some relatively stable and other more variable components, while sex and skeletal configurations may play an important

role.

2. Facial profile shows great variability and low concordance through growth, even among genetically-identical twins.
3. Facial concordance between twins is generally greater after growth has ceased.
4. Considerable differences in the facial profile of monozygotic twins can be seen, even at adulthood.

REFERENCES

1. Langlois J, Kalakanis L, Rubenstein A, Larson A, Hallam M, Smoot M. Maxims or myths of beauty? A meta-analytic and theoretical review. Psychol Bull 2000; 126:390-423.
2. Bishara SE. Facial and dental changes in adolescents and their clinical implications. Angle Orthod 2000; 70:471-83.
3. Merrifield LL. The profile line as an aid in critically evaluating facial esthetics. Am J Orthod 1966; 52:804-22.
4. Bishara SE, Hession TJ, Peterson LC. Longitudinal soft-tissue profile changes: a study of three analyses. Am J Orthod 1985; 88:209-23.
5. Barnette KG. A longitudinal cephalometric study of the soft tissue profile of male and female orthodontically treated Class I and Class II Subjects [dissertation]. St. Louis, MO: St. Louis University; 2008.
6. Primozic J, Perinetti G, Contardo L, Ovsenik M. Facial soft tissue changes during the pre- pubertal and pubertal growth phase: a mixed longitudinal laser-scanning study. Eur J Orthod 2016; 39:1-9.
7. Björk A. Facial growth in man, studied with the aid of metallic implants. Acta Odontol Scand 1955; 13:9-34.
8. Stoner MM, Lindquist JT. A Cephalometric Evaluation of Fifty-Seven Consecutive Cases Treated by Dr. Charles H. Tweed. Angle Orthod 1956; 26:68-98.
9. Riedel RA. An analysis of dentofacial relationships. Am J Orthod 1957; 43:103-119.
10. Subtelny JD. A longitudinal study of soft tissue facial structures and their profile characteristics, defined in relation to underlying skeletal structures. Am J Orthod 1959; 45:481-507.
11. Carlson DS. Theories of craniofacial growth in the postgenomic era. Semin Orthod 2005; 11:172-183.

12. Williams SE, Slice DE. Regional shape change in adult facial bone curvature with age. Am J Phys Anthropol 2010; 143:437-447.
13. Liu F, van der Lijn F, Schurmann C, Zhu G, Chakravarty MM, Hysi PG, et al. A genome- wide association study identifies five loci influencing facial morphology in Europeans. PLoS Genet 2012; 8:e1002932.
14. Paternoster L, Zhurov AI, Toma AM, Kemp JP, St Pourcain B, Timpson NJ, et al. Genome- wide association study of three-dimensional facial morphology identifies a variant in PAX3 associated with nasion position. Am J Human Genet 2012; 90:478-485.
15. Townsend GC, Richards L, Hughes T, Pinkerton S, Schwerdt W. The value of twins in dental research. Aust Dent J 2003; 48:82-8.
16. Manfredi C, Martina R, Grossi GB, Giuliani M. Heritability of 39 orthodontic cephalometric parameters on MZ, DZ twins and MN-paired singletons. Am J Orthod Dentofacial Orthop 1997; 111:44-51.
17. Peng J, Deng H, Cao C, Ishikawa M. Craniofacial morphology in Chinese female twins: a semi-longitudinal cephalometric study. Eur J Orthod 2005; 27:556-61.
18. Šidlauskas M, Šalomskienė L, Andriuškevičiūtė I, Šidlauskienė M, Labanauskas Ž, Vasiliauskas A, Kupčinskas L, Juzėnas S, Šidlauskas A. Heritability of mandibular cephalometric variables in twins with completed craniofacial growth. Eur J Orthod 2016; 38:493-502.
19. Djordjevic J, Zhurov AI, Richmond S; Visigen Consortium. Genetic and Environmental Contributions to Facial Morphological Variation: A 3D Population-Based Twin Study. PLoS One 2016; 11:e0162250.
20. Carels C, Van Cauwenberghe N, Savoye I, Willems G, Loos R, Derom C, Vlietinck R. A quantitative genetic study of cephalometric variables in twins. Clin Orthod Res 2001; 4:130-40.
21. Nanda RS, Meng H, Kapila S, Goorhuis J. Growth changes in the soft tissue facial

profile. Angle Orthod 1990; 60:177-90.

22. Kau CH, Richmond S. Three-dimensional analysis of facial morphology surface changes in untreated children from 12 to 14 years of age. Am J Orthod Dentofacial Orthop. 2008; 134:751-60.
23. Moorrees CFA. Cephalometric appraisal of the face: from the 2-dimensional to the 3- dimensional reconstruction. Rev Orthop Dento Faciale 2000; 34:291-310.
24. Jorde LB, Carey JC, Bamshad MJ. Medical Genetics. 4th ed. Philadelphia, PA: Elsevier; 2009.
25. Bergman RT, Waschak J, Borzabadi-Farahani A, Murphy NC. Longitudinal study of cephalometric soft tissue profile traits between the ages of 6 and 18 years. Angle Orthod 2014; 84:48-55.
26. Lin LI. A concordance correlation coefficient to evaluate reproducibility. Biometrics 1989; 45:255-268.
27. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986; 1:307-310.
28. Dahlberg G. Statistical methods for medical and biological students. London, United Kingdom: George Allen & Unwin; 1940.
29. Tzortzopoulou M. Longitudinal soft tissue profile changes in the untreated Class I and Class III individual: a cephalometric study [dissertation]. St. Louis, MO: St. Louis University; 2009.
30. Nanda SK. Patterns of vertical growth in the face. Am J Orthod Dentofac Orthop 1988; 93:103-16.
31. Eli JT. A longitudinal study of the soft-tissue profile and growth of the nose from childhood though adulthood [dissertation]. St. Louis, MO: St. Louis University; 2005.
32. Behrents RG. An atlas of growth in the aging craniofacial complex. Monograph 18. Craniofacial Growth Series. Ann Arbor: Center for Human Growth and Development;

University of Michigan; 1985.

33. Pecora NG, Baccetti T, McNamara JA Jr. The aging craniofacial complex: a longitudinal cephalometric study from late adolescence to late adulthood. Am J Orthod Dentofacial Orthop 2008; 134:496-505.

34. Sforza C, Grandi G, Binelli M, Dolci C, De Menezes M, Ferrario VF. Age- and sex-related changes in three-dimensional lip morphology. Forensic Sci Int 2010; 200:182.e1-7.

35. Sforza C, Dolci C, Gibelli DM, Codari M, Pucciarelli V, Ferrario VF, Elamin F. Age-related and sex-related changes in the normal soft tissue profile of native Northern Sudanese subjects: a cross-sectional study. Br J Oral Maxillofac Surg 2016; 54:192-7.

36. Rabe-Hesketh S, Skronal A. Multilevel and Longitudinal Modeling Using Stata. 2nd ed. College Station, TX: Stata Press; 2008

37. Pam A, Kemker SS, Ross CA, Golden R. The "equal environments assumption" in MZ–DZ twin comparisons: an untenable premise of psychiatric genetics? Acta Genet Med Gemel 1996; 45:349-60.

38. Aarts BE, Convens J, Bronkhorst EM, Kuijpers-Jagtman AM, Fudalej PS. Cessation of facial growth in subjects with short, average, and long facial types – Implications for the timing of implant placement. J Craniomaxillofac Surg. 2015 Dec;43(10):2106-11

39. Ochoa BK, Nanda RS. Comparison of maxillary and mandibular growth. Am J Orthod Dentofacial Orthop. 2004 Feb;125(2):148-59.

40. Meng HP, Goorhuis J, Kapila S, Nanda RS. Growth changes in the nasal profile from 7 to 18 years of age. Am J Orthod Dentofacial Orthop. 1988 Oct;94(4):317-26.

41. Harris EF, Johnson MG. Heritability of craniometric and occlusal variables a longitudinal sib analysis. Am J Orthod Dentofac Orthop 1991; 99:258-68.

42. Toma AM. Characterization of normal facial features and their association with genes [dissertation]. Cardiff: Cardiff University; 2014.

43. Amini F, Borzabadi-Farahani A. Heritability of dental and skeletal cephalometric

variables in monozygous and dizygous Iranian twins. Orthod Waves 2009; 68:72-79.

44. Dudas M, Sassouni V. The hereditary components of mandibular growth, a longitudinal twin study. Angle Orthod 1973; 43:314-22.

45. Djordjevic J, Jadallah M, Zhurov AI, Toma AM, Richmond S. Three-dimensional analysis of facial shape and symmetry in twins using laser surface scanning. Orthod Craniofac Res 2013; 16:146-60.

46. Baydaş B, Erdem A, Yavuz I, Ceylan I. Heritability of facial proportions and soft-tissue profile characteristics in Turkish Anatolian siblings. Am J Orthod Dentofacial Orthop 2007; 131:504-9.

FIGURE LEGENDS

Fig 1. Cephalometric measurements used in this study: (a) facial convexity angle excluding the nose (soft tissue nasion–subnasale–soft tissue pogonion, in °); (b) nasal prominence (pronasale to soft tissue nasion–subnasale, in mm), (c) nasolabial angle (pronasale–subnasale–labialis superior, in °), (d) upper lip length (subnasale–stomion, in mm), (e) upper lip thickness (labialis superior–S line, in mm), and (f) soft tissue chin thickness (pogonion–soft tissue pogonion, in mm).

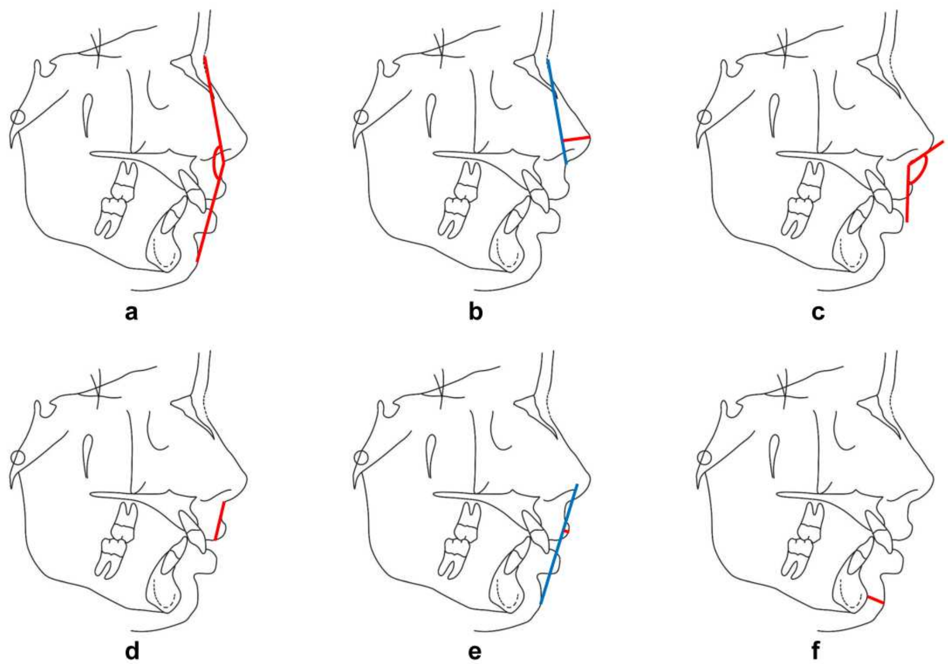

Fig 2. Predictive change curve of the fitted model for differences in facial angle (a; top) and nasal prominence (b; bottom) between the monozygotic twins of each pair, incorporating both fixed- and random-effects.

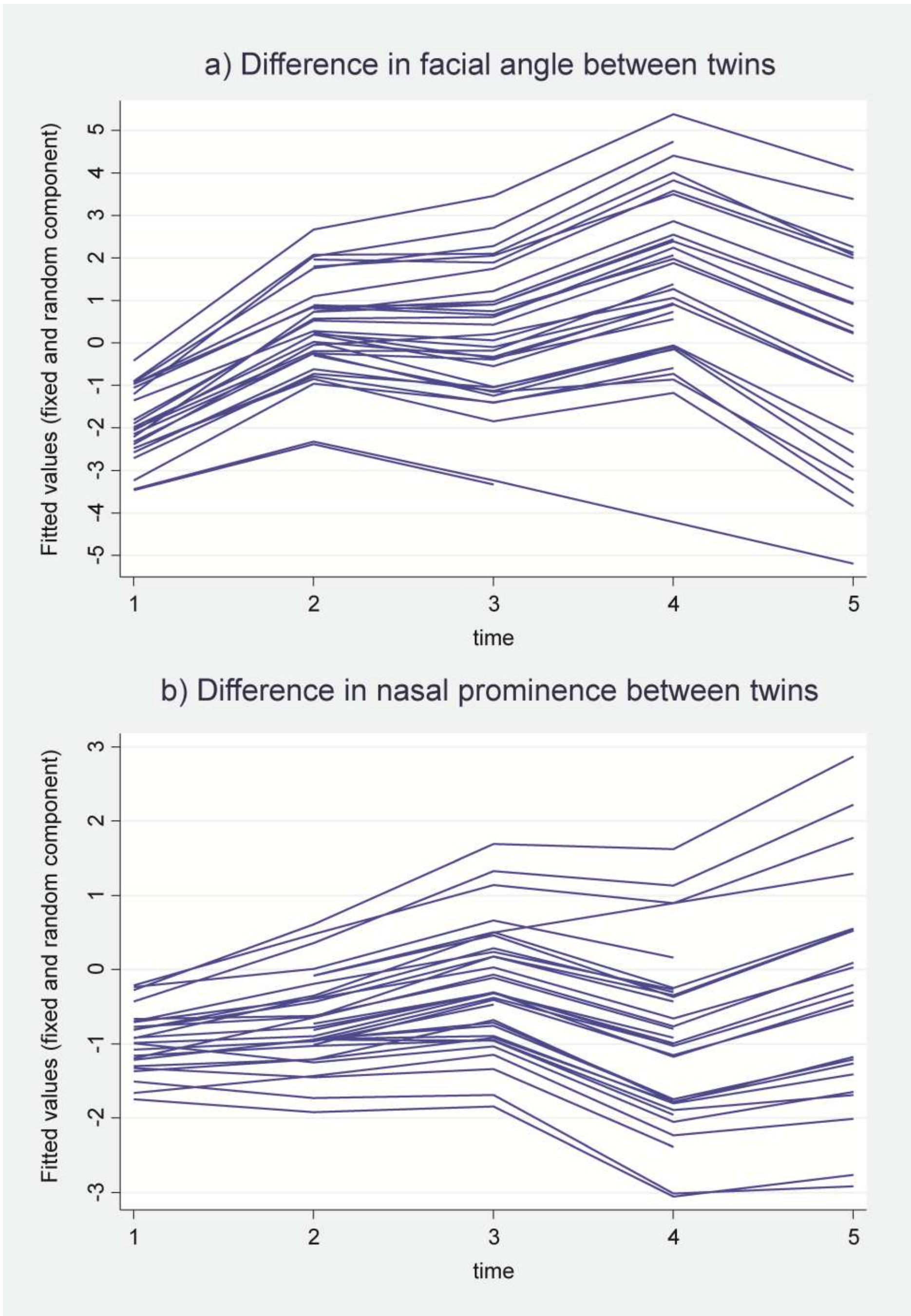

Fig 3. Predictive change curve of the fitted model for differences in nasolabial angle (a; top) and upper lip length (b; bottom) between the monozygotic twins of each pair, incorporating both fixed- and random-effects.

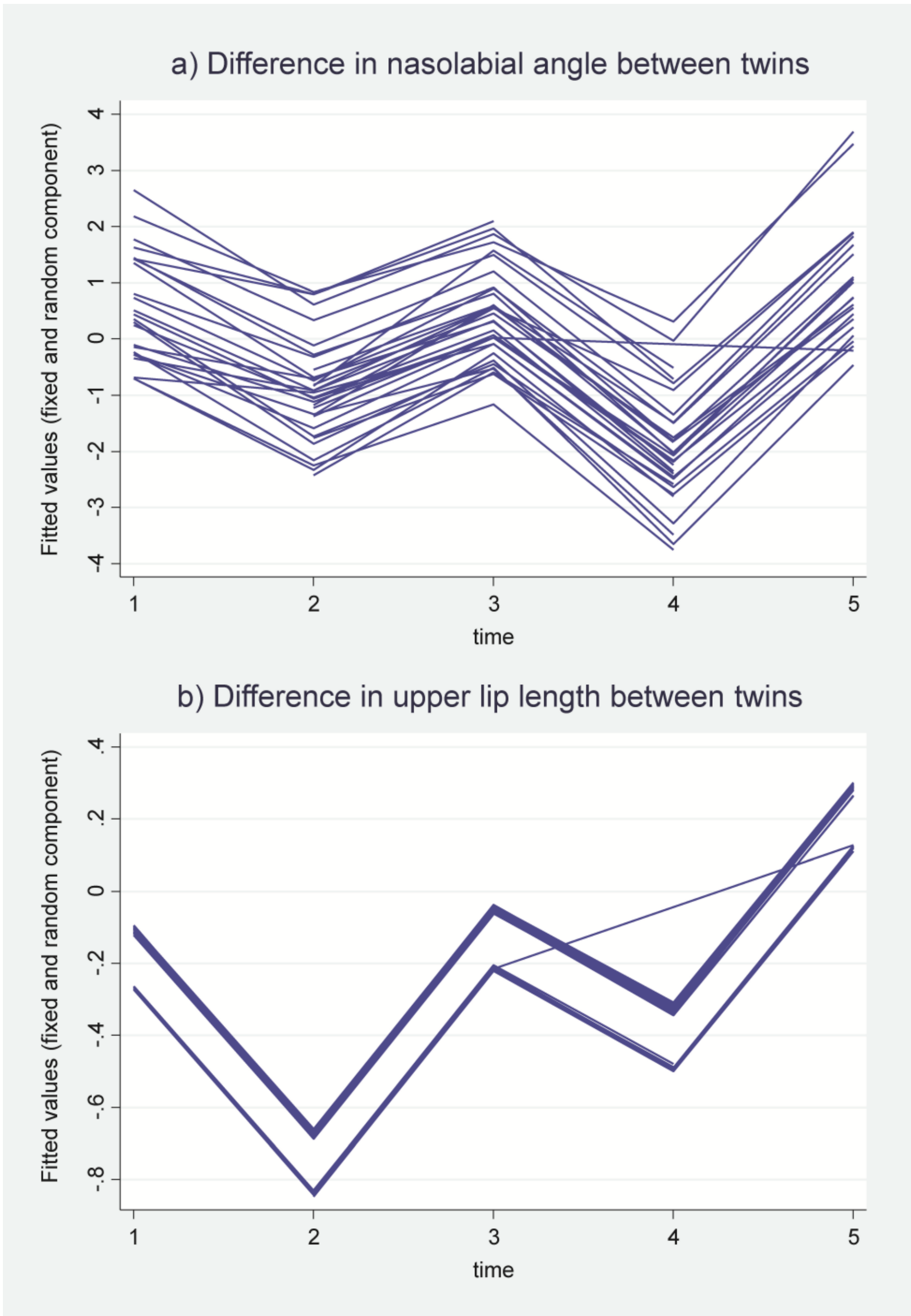

Fig 4. Predictive change curve of the fitted model for differences in upper lip thickness (a; top) and soft tissue chin thickness (b; bottom) between the monozygotic twins of each pair, incorporating both fixed- and random-effects.